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## Recombinant Gal $\alpha$ 1,3Gal-Substituted Mucin/Immunoglobulin Chimeras: A Superior Absorber of Anti-Pig Antibodies

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**P**REVIOUSLY, we have described the construction and production of a recombinant mucin (P-selectin glycoprotein ligand-1)/immunoglobulin fusion protein (patent pending) that became heavily substituted with Gal $\alpha$ 1,3Gal epitopes following co-expression with the porcine  $\alpha$ 1,3 galactosyltransferase in COS cells. We could show that approximately 300 ng of PSGL1/mIgG<sub>2b</sub> immobilized on agarose beads could remove all pig endothelial cell cytotoxic antibodies from 1 mL of pooled human AB serum.

Human AB serum was absorbed on agarose beads carrying PSGL1/mIgG<sub>2b</sub> or pig thyroglobulin (Sigma), or on Gal $\alpha$ 1,3Gal covalently linked via a flexible poly[N-(2-hydroxyethyl)acrylamide] spacer to macroporous glass beads (MPG; Synthesome). Pig endothelial cell cytotoxicity of absorbed and nonabsorbed human AB serum was assessed in <sup>51</sup>Cr release assays. The number of Gal $\alpha$ 1,3Gal epitopes carried on PSGL1/mIgG<sub>2b</sub> was estimated based on the staining intensity of *Bandereia simplicifolia* IB<sub>4</sub> in Western blots using pig thyroglobulin as reference.

Two hundred microliters of agarose beads carrying  $\approx$ 200 ng ( $\approx$ 1 pmol/L) of PSGL1/mIgG<sub>2b</sub> removed more pig

endothelial cell cytotoxic antibodies from 1 mL of pooled human AB serum than did 200  $\mu$ L of agarose beads carrying  $\approx$ 800  $\mu$ g ( $\approx$ 1 nmol/L) of pig thyroglobulin and 100 mg (500  $\mu$ L) of Gal $\alpha$ 1,3Gal-PAA-MPG beads carrying  $\approx$ 85  $\mu$ g ( $\approx$ 250 nmol/L) of Gal $\alpha$ 1,3Gal. PSGL1/mIgG<sub>2b</sub> had six times more BSA IB<sub>4</sub>-reactive epitopes than pig thyroglobulin based on densitometric analysis of BSA IB<sub>4</sub>-stained Western blots.

Finally, PSGL1/mIgG<sub>2b</sub> is a very effective absorber of anti-pig antibodies—an effectiveness that cannot solely be explained by the higher number of Gal $\alpha$ 1,3Gal epitopes expressed on this protein. Instead, additional structural features of the protein and its oligosaccharide side chains must contribute to its effectiveness.

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